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柬埔寨龙血树褐斑病的病原鉴定及其培养条件分析

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摘　要:[目的]柬埔寨龙血树为我国各省市广泛栽培的室内观叶植物,近年来普遍发生叶部病害影响其观赏价值。2011年8月至2016年12月,在云南省蒙自市、新疆乌鲁木齐的柬埔寨龙血树发现一种叶部初期褪绿逐渐变为灰褐色凹陷最后暗褐色枯死的褐斑病。为了确定其病原菌种类和发病条件,进行病原菌鉴定及培养条件测试。[方法]采用在云南蒙自、新疆乌鲁木齐采集的12份柬埔寨龙血树褐斑病病叶,开展了组织分离、致病性测试、形态学观察、分子鉴定与培养条件测试。[结果]从12份病叶分离到15株真菌,菌落中央产生黑色孢子,边缘白色,顶囊球形或近球形,直径38～45 μm,分生孢子球形暗褐色,直径2～5 μm,代表性菌株LZB－6、LZB－12经CaM或β－tubulin系统发育分析确定为塔宾曲霉;刺伤法接种14 d的龙血树叶片均发病,症状与田间一致,并能分离到菌落形态、分生孢子形态与原分离菌株一致的真菌,完成科赫法则验证确定为病原菌。培养条件测试表明,病原菌菌丝生长的适宜温度为22～34 ℃,最适宜温度为28 ℃,光照抑制菌丝生长,菌丝生长偏爱碱性环境,pH为7～10时,随着pH值升高生长速率仍在增加,最适宜碳源和氮源分别为甘露醇、明胶,菌丝致死温度为55 ℃ 10 min;产孢适宜温度为28～40 ℃,最适宜温度为32 ℃,光照抑制产孢,适宜产孢pH值为6～8,最适宜pH值为8,最适宜碳源和氮源分别为蔗糖、明胶,孢子的致死温度为60 ℃ 10 min。[结论]导致云南蒙自、新疆乌鲁木齐的柬埔寨龙血树褐斑病菌均为塔宾曲霉,除了光照和氮源之外,温度、pH值、碳源对菌丝生长和产孢的影响均不同,22～34 ℃时病原菌能较好的生长与产孢,应加强对柬埔寨龙血树褐斑病的防控。

关键词:柬埔寨龙血树; 褐斑病; 塔宾曲霉; 系统发育分析; 产孢
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龙血树属(*Dracaena* Vand ex L.)大部分原产于非洲与亚洲的热带及亚热带地区,约有50种;我国有6种,柬埔寨龙树(*D. cambodiana*,又称海南龙血树、剑叶龙血树、小花龙血树)、长花龙血树(*D. angustifolia*,又称狭叶龙血树、番仔林投)、矮龙血树(*D. terniflora*)、细枝龙血树(*D. elliptica*)、河口龙血树(*D. houkouensis*)和深脉龙血树(*D. impressivenia*)[1]。此外,我国还有一些引进的其它种的栽培品种或园艺品种,例如普通也门铁(*D. fragrans*)、金心也门铁(*D. fragrans* cv. massangeana)和金边也门铁(*D. fragrans* cv. lindenii)[2]、金边富贵竹(*D. sanderiana* cv. golden edge)、银边富贵竹(*D. sanderiana* cv. celes)、星点木(*D. godseffiana* cv. bausei)、油点木(*D. surculosa* cv. maculata)[1]。龙血树属因为柬埔寨龙血树、龙血树(*D. draco*)等能够产生血竭具有药用价值[3-4]、也门铁与富贵竹具有观叶观花的观赏价值并能吸收有毒有害物质清新空气而广受关注[1,5]。

目前龙血树属植物病害的研究多集中于观赏性树种,已报道香龙血树(*D. fragrans*)及其栽培

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品种病害有炭疽病(*Colletotrichum gloeosporioides*)、小球腔菌褐斑病(*Leptosphaeria draconis*)、可可球二孢叶斑病(*Botryodiplodia theobromae*)、拟茎点霉叶斑病(*Phomopsis dracaenicola*)、弯孢霉灰斑病(*Curvularia lunata*)、弯孢霉褐斑病(*Cur. senegalensis*)、拟盘多毛孢叶斑病(*Pestalotiopsis clavispora*)、拟茎点霉灰斑病(*P. dracaenae*)、球壳孢叶枯病(*Sphaeropsis dracaenae*)、顶多毛孢叶斑病(*Bartalinia dracaenae*)[6-7]、欧式杆菌叶枯病(*Erwinia ananas*)[8],富贵竹(*D. sanderiana*)病害有疫霉叶枯病(*Phytophthora nicotianae*)[9]、泛菌萎蔫病(*Pantoea stewartii*)[10]、炭疽病(*C. dracaenophilum*, *C. gloeosporioides*)[11-12]、镰刀菌茎腐病(*Fusarium solani*, *F. proliferatum*)[13-15],长花龙血树病害有白绢病(*Sclerotium rolfsii*)[16]、镰刀菌根腐病(*F. oxysporum*)[17],大香龙血树(*D. arborea*)病害有可可球二孢叶斑病(*B. heobromae*)、炭疽病(*C. capsici*、*Glomerella cingulata*)、弯孢霉叶斑病(*Curvularia* sp.)、小球腔菌叶枯病(*L. agaves*)、拟茎点霉叶斑病(*P. dracaenae*、*P. dracaenicola*)[18]、镰刀孢叶斑病(*F. oxysporum*)[19],千年木(*D. marignata*)病害有链格孢叶斑病(*Alternaria arborescens*)[20],*D. aletriformis*病害有曲霉叶斑病(*Aspergillus terreus*)[21]。

2011年8月至2016年12月,在云南蒙自文澜镇与新安所镇、新疆乌鲁木齐的花鸟市场调查发现,柬埔寨龙血树幼嫩叶片的叶尖与叶缘先是褪绿,继而变为灰褐色,病斑凹陷(图1A),最后变为暗褐色、枯死,干燥时病斑中央灰白色并沿叶脉纵向破裂、穿孔,云南蒙自文澜镇调查约1000棵,发病率100%;新疆乌鲁木齐调查约200棵,发病率30%。该病害与龙血树属已报道病害病症均不相同,因此本研究通过组织分离、形态学与分子生物学分析及致病性测试与再分离,确认褐斑病的病原菌分类学地位,继而测试温度、pH值、光照及不同碳氮源对病原菌菌丝生长与产孢量的影响,旨在为该病害的防控提供理论依据。

1 材料和方法

1.1 试验材料

2012年6月-2013年12月,从云南省红河州蒙自市文澜镇、新安所镇各采集3株发病植株的病叶,计6份样品;2014年10月-2015年4月,从新疆维吾尔自治区乌鲁木齐新市区植物园花卉市场、沙依巴克区明珠花卉市场各采集3株发病植株的病叶,共计6份样品。科赫法则验证致病性所用无病龙血树,购买自明珠花卉市场。

马铃薯葡萄糖琼脂(PDA)培养基:削皮马铃薯200.00 g、葡萄糖20.00 g、琼脂粉15.00 g,蒸馏水定容至1000.00 mL,pH自然[22]。

改良沙氏葡萄糖蛋白胨琼脂培养基(GSDA):蛋白胨10.00 g,葡萄糖20.00 g,琼脂粉15.00 g,水1000 mL,pH自然;参照周光燕等[23]研究方法并修改,葡萄糖由25.00 g改为20.00 g,蛋白胨由1.00g改为10.00 g,去除0.50 g酵母膏,琼脂粉由20.00 g改为15.00 g,pH自然。

1.2 试验方法

1.2.1 菌株分离与纯化

采用组织分离法分离病原菌[24-25]并略加改良,无菌水冲洗叶片3次,每份病叶剪取带有病斑的大小为5 mm × 5 mm的组织块,在超净台中浸入75%乙醇消毒1 min,转入0.1%次氯酸钠溶液消毒2 min,无菌水冲洗3次,以无菌滤纸吸干表面水分,转到PDA培养基上28 ℃暗培养2~4 d,根据组织边缘菌丝的致密程度、颜色等特征从不同形态的菌落边缘挑取菌丝尖转接到新鲜PDA培养基,多次转接纯化。

1.2.2 致病性测试与再分离

采用无菌水洗脱28 ℃暗培养15 d的分离菌株孢子,4层擦镜纸过滤,将孢子悬液调节到10^6个·mL^{-1}备用。采用针刺点接法接种[22],从长势一致的龙血树顶部选取大小接近的叶片,每株选择不同方向的5个叶片,先用无菌水冲洗表面的灰尘,然后用75%乙醇擦拭5遍,再用无菌水冲洗3遍,自然晾干。以无菌接种针刺伤叶片,每片叶子刺伤处滴加60 μL孢子悬浮液,每个菌株接种3株龙血树15片叶子;以针刺滴加60 μL无菌水的3株龙血树15片叶子作为对照。将湿润的无菌脱脂棉覆盖在叶子接种处,覆盖保鲜膜保湿,置于20~25 ℃的温室,相对湿度保持在90%以上,3 d后除去保鲜膜和脱脂棉,逐日观察并记录发病情况。发病后,每株选取1片病叶,按照1.2.1描述方法进行菌株再分离,菌落与分生孢子特征与出发菌株一致的视为病原菌。

1.2.3　形态学观察

参照张阳等[26]描述的方法,将供试菌株转接在PDA培养基上,25 ℃暗培养7 d,以无菌接种针挑取菌丝制备临时玻片,在奥林巴斯BX51显微镜和Imager Z1显微镜数码成像系统拍摄分生孢子梗、分生孢子的形态,测量并记录50个顶囊、分生孢子的大小。

1.2.4　分子鉴定

用灭菌竹签刮取PDA培养基上25℃暗培养7 d的代表菌株菌丝体,根据FASTPREP-®24组织破碎仪(美国MP公司)和索莱宝真菌基因组提取试剂盒(北京索莱宝科技有限公司)使用说明书提取DNA。分别采用用CaM和β-tubulin基因序列的通用引物CMD5(5′-CCGAGTACAAGGARG-CCTTC-3′)/CMD6(5′-CCGATRGAGGTCAT-RACGTGG-3′)、Bt2a(5′-GGTAACCAAATCG-GTGCTGCTTTC-3′)/Bt2b(5′-ACCCTCAGT-GTAGTGACCCTTGGC-3′)进行PCR扩增[27-28]。PCR扩增采用50 μL体系:5 μL 10×PCR Buffer(含100 mM $MgCl_2$,10 mM 二巯基丁二醇和500 mM NaCl)、*Taq* DNA聚合酶2.5 U、10 mmol·L^{-1} dNTP 1 μL、15 ng模板DNA、10 μmol·L^{-1}各0.5 μL,无菌超纯水补足至50 μL。反应程序为94 ℃预变性4 min;94 ℃变性1 min,56 ℃退火1 min,72 ℃延伸1 min,35个循环;72 ℃最终延伸10 min,4 ℃保存[29]。PCR产物经TaKaRa琼脂糖凝胶DNA纯化试剂盒Ver.4.0[宝生物工程(大连)有限公司]纯化,纯化后的PCR产物送生工生物工程(上海)股份有限公司进行测序,用Bioedit 5.0软件和BLAST进行多序列比对分析,提交序列至GenBank。下载*Aspergillus* 12个菌株的β-tubulin、8个菌株的CaM序列,以*Talaromyces flavus*作为外群,采用MEGA 6.0中的最大似然法(maximum likelihood,ML)构建系统发育树[30]。

1.2.5　培养条件测试

参考杨丽芬等[31]描述的方法,将代表菌株在PDA培养基上28 ℃恒温暗培养3 d,用直径5.00 mm的打孔器在菌落边缘打取菌饼,分别置于不同温度(20、22、24、26、28、30、32、34、36、38、40 ℃,暗培养)、不同光照(28 ℃全光照培养、28 ℃全暗培养和12小时黑暗交替28 ℃)、不同pH值(5、6、7、8、9、10,28℃暗培养)、不同碳源(以20.00 g·L^{-1}的木糖、果糖、甘露醇、麦芽糖、山梨醇、蔗糖、可溶性淀粉7种碳源代替改良沙氏葡萄糖蛋白胨琼脂基培养基中的葡萄糖,改良沙氏葡萄糖蛋白胨琼脂基培养基的葡萄糖为对照,28 ℃暗培养)、不同氮源(以10.00 g·L^{-1}的硝酸铵、硝酸钾、硫酸铵、磷酸铵、牛肉膏、尿素、明胶、甘氨酸、甲流氨酸、谷氨酸10种氮源代替改良沙氏葡萄糖蛋白胨琼脂培养基中的蛋白胨,改良沙氏葡萄糖蛋白胨琼脂基培养基的蛋白胨为对照,28 ℃暗培养),每个处理重复3次。培养3 d后按照十字交叉法测菌落直径,以血球计数板法测试孢子产量。

将5 mm的菌饼加入盛有3 mL无菌水的试管中,另取10^6个·mL^{-1}的孢子悬液1 mL加入盛有9 mL无菌水的试管中震荡混匀,40、45、50、55、60、65 ℃恒温水浴10 min,转入自来水中冷却至室温,取出菌饼以无菌滤纸吸干水分,转到新鲜PDA培养基上28 ℃暗培养5 d;或吸取100 μL孢子悬液涂布新鲜PDA培养基上28 ℃暗培养5 d,观察菌饼或孢子是否萌发新菌丝。

1.3　统计分析

用SPSS 17.0和Excel 2010进行统计分析和制图,以"平均数±标准差"表示菌落直径大小,用Duncan多重比较法进行差异显著性检验。

2　结果与分析

2.1　菌株分离

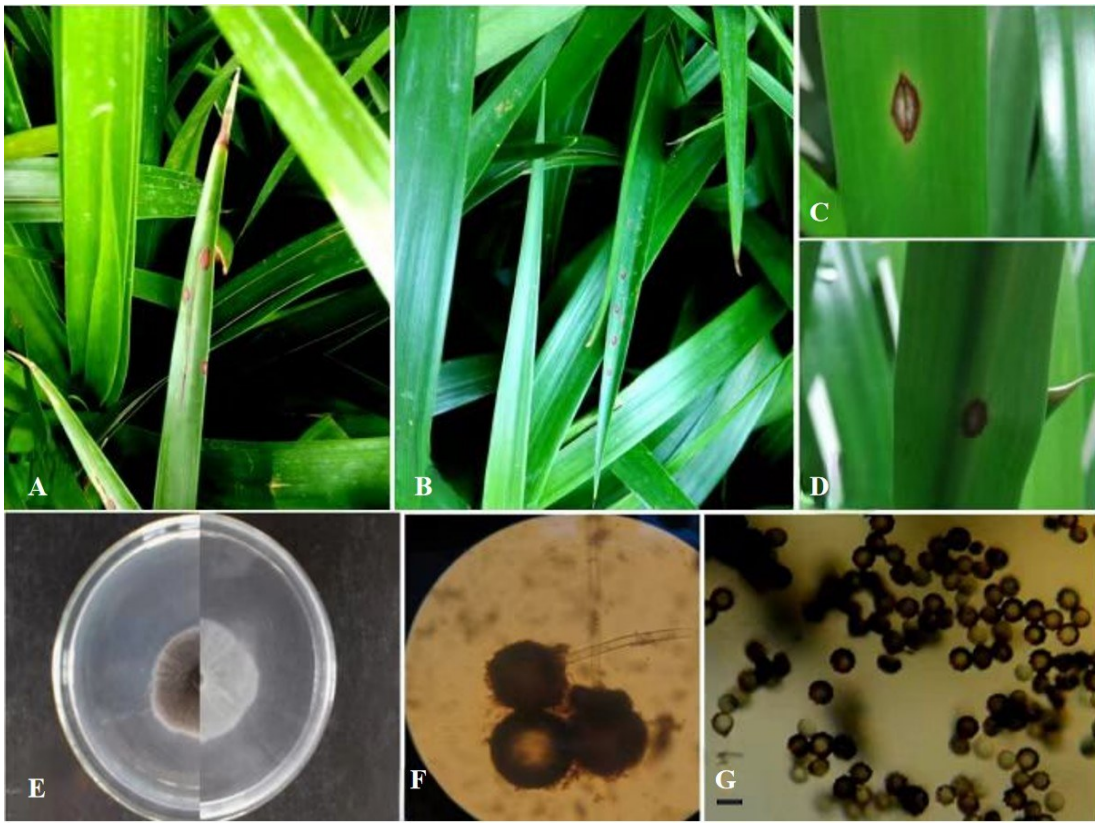
从云南蒙自6份病害样品分离获得6株真菌,依次编为LZB-1～LZB-6;从新疆乌鲁木齐6份病害样品分离获得9株真菌,依次编为LZB-7～LZB-15,其菌落宏观形态与分生孢子形态基本一致,根据形态学初步鉴定为*Aspergillus* sp.。

2.2　致病性测试与再分离

接种14 d的龙血树叶片均发病,发病初期针刺伤口周围出现水浸状的暗褐色斑点,继而凹陷,最后变为暗褐色(图1B);停止加湿后,病斑中央变为灰白色并沿叶脉纵向破裂穿孔(图1C-D),并能从病害组织分离到菌落形态、分生孢子形态与出发菌株一致的真菌。而以无菌水接种的对照不发病,并且不能从针刺伤口处分离到与出发菌株一致的真菌。

2.3　病原菌鉴定

云南蒙自菌株LZB-6、新疆乌鲁木齐菌株



A:温室中柬埔寨龙血树褐斑病的症状;B:LZB-12接种导致的病害症状;C:接种LZB-12的龙血树叶子正面病斑;D:接种LZB-12的龙血树叶子反面病斑;E:LZB-12菌落;F:LZB-12顶囊;G:LZB-12的分生孢子,比例尺=5 μm

A: The symptom of brown leaf spot on *D. cambodiana* in commercial greenhouses; B: The disease symptom caused by isolate LZB-12; C: A lesion on the adaxial leaf surface of *D. cambodiana* caused by isolate LZB-12; D: A lesion on the adaxial dorsal leaf surface of *D. cambodiana* caused by isolate LZB-12; E: The colony of LZB-12; F: The vesicle of LZB-12; G: The conidia of isolate LZB-12, Bar=5 μm

图1 塔宾曲霉的致病症状与形态学特征

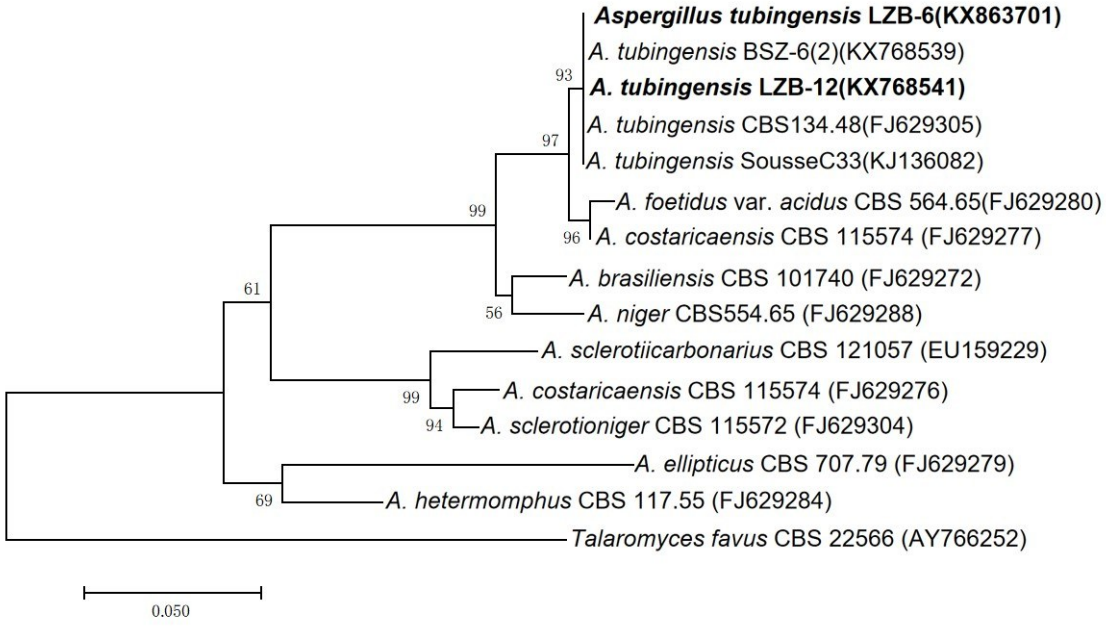
Fig.1 Disease symptoms and morphological features of *Aspergillus* sp.

LZB-12接种在PDA培养基上28 ℃暗培养,初期菌丝白色,菌丝紧贴培养基生长,3 d后接种点及其边缘出现黑色粉状物,菌落直径6.00~7.00 cm;7 d长满90 mm直径培养皿,菌落边缘白色,其余均布满黑色粉状物。顶囊球形或近球形,黄色,直径38~45 μm;表面覆盖绿色瓶梗,瓶梗长24~32 μm;瓶梗上链生球形的暗褐色分生孢子,分生孢子直径2~5 μm,表面光滑或布满小刺。这与塔宾曲霉*Aspergillus tubingensis*(Schober)Moss的形态学[32]一致。

云南蒙自菌株LZB-6用通用引物Bt2a/Bt2b扩增后获得528 bp的β-tubulin基因序列;新疆乌鲁木齐菌株LZB-12用通用引物Bt2a/Bt2b扩增后获得530 bp的β-tubulin基因序列,用真菌通用引物CMD5/CMD6扩增后分别获得586 bp的CaM基因序列。将其序列登录NCBI网站(Http://blast.ncbi.nlm.nih.gov/Blast.cgi)进行Blast分析,LZB-12的CaM基因序列(KX768540)与*A. tubingensis* BSZ-6(2)(KX768538)的序列相似性达100%,LZB-6与LZB-12的β-tubulin基因序列(KX863701、KX768541)与*A. tubingensis* SousseC33(KJ136082)、BSZ-6(2)(KX768539)的序列相似性均达100%。基于β-tubulin基因序列构建的系统发育树表明,LZB-6与LZB-12与中国云南小桐子叶斑病*A. tubingensis* isolate BSZ-6(2)[30]、突尼斯中性粒细胞减少症病原菌*A. tubingensis* SousseC3及*A. tubingensis* strain CBS 134.48聚在一个分支(图2);基于CaM基因序列构建的系统发育树表明,LZB-12与捷克人体病原菌*A. tubingensis* culture collection CCF<CZE>:4086、尼日利亚食品微生物*A. tubingensis* isolate NRRL 62643等聚在一个分支(图3)。同源比对与系统发

育分析均支持菌株LZB-6、LZB-12均为塔宾曲霉*A. tubingensis*(Schober) Moss。

因而,基于形态学与分子鉴定结果,确认云南、新疆柬埔寨龙血树褐斑病的病原菌为塔宾曲霉*A. tubingensis*(Schober) Moss。

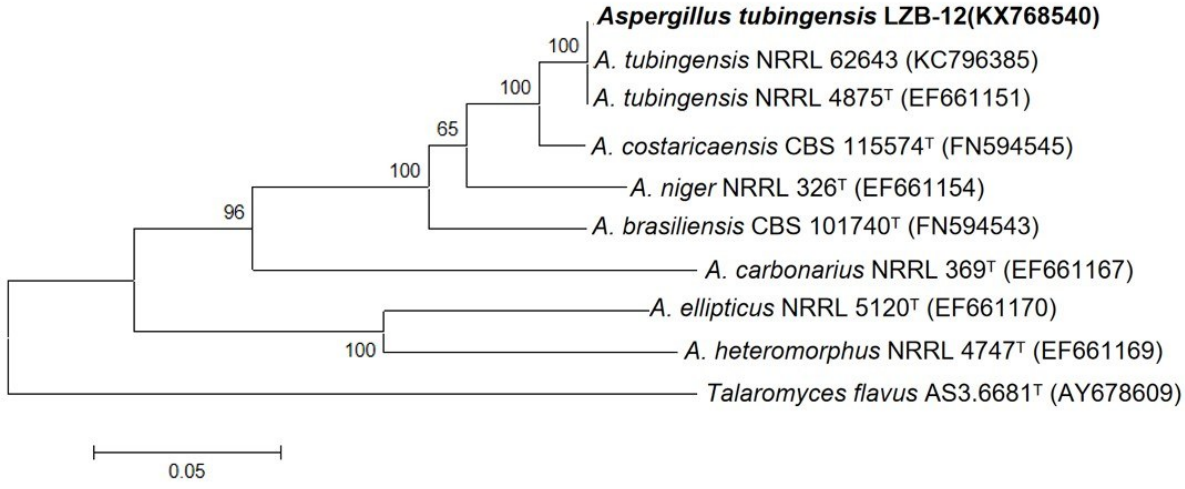


褐斑病病原菌LZB－6、LZB－12与曲霉属相关分类单元的关系,*Talaromyces flavus*作为外群;分支节点表示1000次重复的Bootstrap值大于50%,比例尺表示每个核苷酸位置0.05的变化

The relationship of LZB－6 and LZB－12 with other 12 species of *Aspergillus*; *Talaromyces flavus* was used as the outgroup species; Numbers at the nodes indicate the level of Bootstrap values (50 %) based on 1000 replications. *Bar* represents 0.05 changes per nucleotide position

图2　基于曲霉属12个菌株与LZB-6、LZB-12的β-tubulin序列构建的系统发育树

Fig. 2　Phylogenetic tree constructed with the β-tubulin sequences of 12 *Aspergillus* isolates retrieved and isolate lzb-6 and LZB-12



*Talaromyces flavus*作为外群;分支节点表示1000次重复的Bootstrap值大于50%;比例尺表示每个核苷酸位置0.05的变化

Talaromyces flavus was used as the outgroup species; Numbers at the nodes indicate the level of Bootstrap values (50 %) based on 1000 replications; *Bar* represents 0.05 changes per nucleotide position

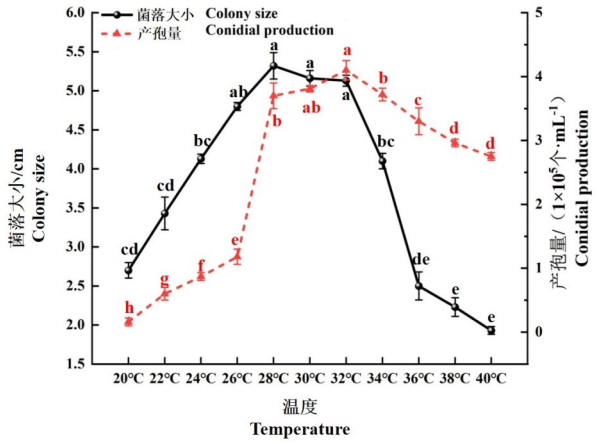
图3　基于曲霉属9个菌株与LZB-12的CaM序列构建的系统发育树

Fig.3　Phylogenetic tree constructed with CaM sequences of 9 *Aspergillus* isolates and isolate LZB-12

2.4　病原菌培养条件

在20～40 ℃菌株LZB-6菌丝生长和产孢均呈现先增高后下降的趋势,并均能生长、产孢。22～34 ℃之间LZB-6在3 d后的菌落直径均3.40 cm

以上,生长速度显著高于20 ℃与34～40 ℃,最适合温度28 ℃时菌落直径达5.32 cm;28～40 ℃产孢量均在2.70×10^{5}个·mL^{-1}以上,显著高于20～26 ℃,最适合温度32 ℃时产孢量达4.10×10^{5}个·mL^{-1}。



差异显著性分析采用Duncan新复极差法,取a=0.05为差异显著水平,同一指标字母相同代表它们之间差异不显著,同一指标字母不相同代表它们之间差异显著。菌落直径、产孢量均为培养3 d后的测量值。下同

Duncan's new multiple range test was used for the analysis of significant differences, with a=0.05 as the level of significant differences. The same letters in the same index indicated that the differences between them were not significant, while the different letters in the same index indicated that the differences between them were significant. The colony diameter and spore production were measured after 3 days of cultivation. The same conditions applied below

图4 温度对菌丝生长及产孢量的影响

Fig.4 Effects of temperature on mycelia diameter and spores yield

光照对云南蒙自菌株LZB-6菌丝生长与产孢量的影响不同,3种光照条件下菌落直径差异不显著,28 ℃暗培养3 d后的菌落直径在5.06～5.26 cm之间;光照抑制病原菌产孢,3种条件下产孢量差异显著,28 ℃暗培养3 d后的产孢量为3.70×10^{5}个·mL^{-1}。

酸碱度对云南蒙自菌株LZB-6菌丝生长与产孢的影响不同,在pH值5～10之间,菌落直径呈现上升趋势并均在4.00 cm以上,即病原菌营养生殖偏爱碱性环境;该菌均能产孢,差异显著,pH值6～8适宜产孢,最适宜pH值为8时产孢量为3.53×10^{5}个·mL^{-1}。

云南蒙自菌株LZB-6菌丝生长与分生孢子产生对碳源的需求不同,在包括葡萄糖在内的8种培养基上均能生长、产孢,其中甘露醇、木糖、山梨醇、麦芽糖、果糖、可溶性淀粉比较适应生长需求,菌落直径均在3.20 cm以上,在最佳碳源甘露醇为碳源的培养基上菌落直径为3.73 cm;蔗糖、甘露

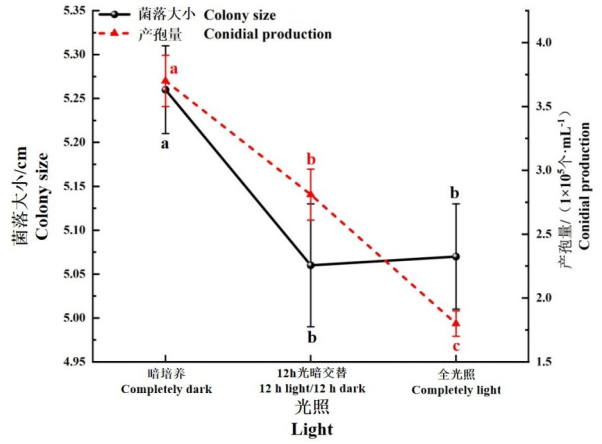


图5 光照对病原菌菌丝生长及产孢量的影响

Fig.5 Effects of light density on mycelia diameter and spores production

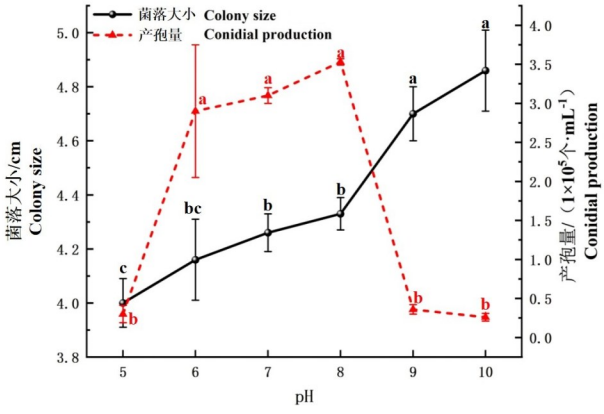


图6 pH对病原菌菌丝生长及产孢量的影响

Fig.6 Effects of pH value on mycelia diameter and spores production

醇、麦芽糖、木糖比较适应产孢需求,在最佳碳源蔗糖为碳源的培养基上产孢量为1.30×10^{5}个·mL^{-1}。

云南蒙自菌株LZB-6菌丝生长与产孢对氮源的需求也不相同,在包括蛋白胨在内的11种培养基上均能生长、产孢,但明胶、硝酸钾均比较适应菌丝生长、产孢需求,菌落直径均在3.16 cm以上,产孢量也在0.64×10^{5}个·mL^{-1},最佳氮源均为明胶。

经不同温度水浴处理10 min的云南蒙自菌株LZB-6菌丝块,40～50 ℃处理10 min的菌丝块转移到新鲜的PDA培养基后5 d仍能生长,55～65 ℃处理过的菌丝块转移到新鲜培养基后停止生长;40～55 ℃处理10 min的孢子涂布在新鲜培养基后5 d仍能萌发生长形成菌落,60与65 ℃处理10 min的孢子不能萌发,表明该菌的菌丝致死温度为55℃ 10 min,孢子的致死温度为60 ℃ 10 min(表1)。

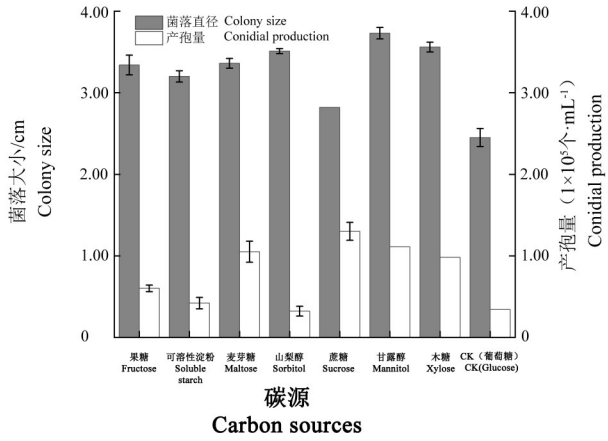


图7　碳源对病原菌菌丝生长及产孢量的影响

Fig. 7　Effects of carbon sources on colony diameter and spores production

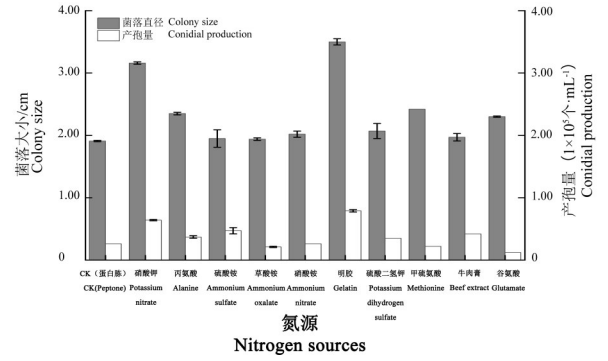


图8　氮源对病原菌菌丝生长及产孢量的影响

Fig. 8　Effects of nitrogen sources on mycelia diameter and spores production

表1　菌丝及孢子致死温度

Table 1　The lethal temperature of mycelia and conidia

处理温度/℃ Treated temperature	5 d后菌丝生长情况 Mycelia growth of 5 d after treated	5 d后孢子萌发情况 Conidial germination of 3 d after treated
40	生长	萌发
45	生长	萌发
50	生长	萌发
55	不生长	萌发
60	不生长	不萌发
65	不生长	不萌发

3　讨论

本研究通过对云南蒙自、新疆乌鲁木齐柬埔寨龙血树褐斑病的病原菌分离、科赫法则验证、形态学与系统发育学分析,明确了柬埔寨龙血树褐斑病的病原菌为塔宾曲霉,这是塔宾曲霉引起柬埔寨龙血树叶部褐斑病的首次报道。自Guo等[32]报道了塔宾曲霉引起的中国云南蒙自小桐子叶褐斑病以来,福建岩茶赤叶斑病[33]、上海铁筷子属植物叶斑病[34]、巴基斯坦棉花叶斑病[35]、上海不结球白菜叶斑病[36]等叶部病害相继报道由塔宾曲霉引起;此外,近年来还陆续报道了该菌引起的韩国葡萄[37]、美国草莓[38]、中国石榴[39]等多汁、富含糖分水果果腐病。相对以往塔宾曲霉等多引起采后病害[40-42],原因可能是随着传统主要病害抗病品种的选育和各种防控措施的采用,传统病害受到一定的抑制,而以往危害不重关注度较低的塔宾曲霉等弱致病菌导致病害严重程度逐渐上升。

越来越多的研究表明一种病害往往由多种病原菌复合侵染引起,并且不同地区的优势病原菌可能不同,这也将导致防治措施的不同。例如,中国主要粮食和油料作物195种病害中的38种病害由2个及以上的病原菌导致,玉米苗枯病有9种镰刀菌、玉米腐霉根腐病有15种腐霉[43-44];北京桃褐腐病338株病原菌为*Monilinia fructicola*、4株为*M. fructigena*,表明北京桃褐腐病优势病原菌为*M. fructicola*[45]。*A. terreus*能引起龙血树属*D. aletriformis*曲霉叶斑病[21]、塔宾曲霉能引起富贵竹采后病害[45],并且*D. aletriformis*曲霉叶斑病与福建武夷山岩茶赤叶斑病一样病斑中心产生红色斑点,因此不同地区甚至相同地区的柬埔寨龙血树褐斑病病原菌可能由塔宾曲霉在内的多种曲霉组成,尚需要进一步的系统研究。

对柬埔寨龙血树褐斑病菌云南蒙自菌株LZB-6的培养条件测试结果表明,28 ℃菌丝生长最快,光照抑制菌丝生长,偏爱碱性环境,最佳碳氮源分别为甘露醇、明胶,菌丝致死温度为55 ℃处理10 min;产孢最佳温度32 ℃,光照抑制产孢,最佳pH值8,最佳碳氮源分别为蔗糖、明胶,孢子致死温度为55 ℃处理10 min。这与小桐子褐斑病菌菌丝生长、产孢最适温度均为35 ℃,菌丝生长最适pH值为8、产孢最适pH值为6,全光照时菌丝直径和产孢量最大,最佳氮源为牛肉膏不同,而与菌丝生长最佳碳源为甘露醇、产孢最佳碳源为可溶性淀粉与蔗糖接近[47]。这可能与其寄主小桐子常栽培在向阳、干旱贫瘠的山坡而长花龙血树种植在温暖、潮湿的室内有关。柬埔寨龙血树本身喜光,在褐斑病严重时可剪去病叶、放在向阳处并喷施中

性甚至偏酸性农药控制病害发展与病菌产孢。

张阳等[26]测试了温度、湿度、光照等环境条件对病原菌在寄主组织上萌发的影响,杨丽娜等[48]测试了温度、湿度、光照对病原菌孢子侵染桃嫩枝条的影响,陈泉等测试柑橘叶片、果实、枝条成熟度及菌龄对轮斑病菌侵染的影响,并评估了47个柑橘品种对3省来源轮斑病菌的抗性[49-51]。温度、湿度、光照对孢子萌发及对寄主组织致病性的影响研究,有助于了解病菌侵染环境条件;测试菌龄及叶片、果实、枝条成熟度对发病率及病斑扩展的影响,有助于了解病菌的侵染部位及侵染组织龄期,均对制定病害预防的温度、湿度、光照等环境条件及寄主组织的喷雾部位具有重要的现实意义,未来病原菌生物特性研究应当采用温度、湿度、光照对病原菌在寄主组织上萌发、侵染寄主组织的影响,以及菌龄及叶片、果实、枝条成熟度对发病率及病斑扩展的影响替代目前的病原菌培养条件研究。

4 结论

塔宾曲霉传统上多引起采后病害,但近年来引起多汁、富含糖分水果果腐病和叶斑病的报道日益增多。塔宾曲霉产孢量大,可随风和雨水传播,因此有必要加强塔宾曲霉导致的水果与观叶植物叶部病害识别与防治。本研究分离与鉴定了云南蒙自和新疆乌鲁木齐的柬埔寨龙血树褐斑病的病原菌,并提供了田间症状、人工接种症状及分生孢子形态,为该病害的田间诊断及病原菌鉴定提供依据;同时测试了温度、光照、pH值、氮源、碳源对病原菌菌丝生长与产孢的影响,以及菌丝与分生孢子的致死温度,为柬埔寨龙血树褐斑病发生的田间预测与防治提供参考。

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Identification of the pathogen causing brown leaf spot in *Dracaena cambodiana* and analysis of its cultivation conditions

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Abstract: [Objective] *Dracaena cambodiana* is a widely cultivated indoor ornamental plant in various provinces of China. In recent years, a common leaf disease has affected its ornamental value. Between August 2011 and December 2016, a brown spot disease was discovered in the leaves of *D. cambodiana* in Mengzi City, Yunnan Province and Urumqi, Xinjiang. The disease started with the leaves turning chlorotic, gradually becoming grayish-brown, then developing sunken areas, and eventually turn-

ing dark brown and dying. In order to determine the type of pathogen and the conditions under which the disease developed, we conducted pathogen identification and cultivation condition tests. [Methods] A total of 12 samples of Cambodian dragon tree leaves with brown spot disease were collected from Mengzi, Yunnan, and Urumqi, Xinjiang. These samples were used for tissue isolation, pathogenicity testing, morphological observation, molecular identification, and cultivation condition testing. [Results] Fifteen fungal strains were isolated from the 12 diseased leaf samples. The colonies had black spores at the center, with white edges. The spore bodies were spherical or nearly spherical, with a diameter of 38～45 μm. The conidia were dark brown, spherical, and 2～5 μm in diameter. Phylogenetic analysis of representative strains using CaM or β-tubulin confirmed that the pathogen *Aspergillus tubingensis*; Inoculation of the leaves of the dragon tree with the pathogen caused disease within 14 days, and the symptoms matched those observed in the field. Fungi with colony and conidia morphology consistent with the original isolates were re-isolated, confirming the pathogen through Koch′s postulates. Cultivation condition tests showed that the optimal temperature for mycelial growth was 22～34 ℃, with the most suitable temperature being 28 ℃. Light inhibited mycelial growth, and the fungus preferred an alkaline environment. The growth rate increased with a pH range of 7～10, with the best growth at pH 8. The most suitable carbon and nitrogen sources were mannitol and gelatin, respectively. The lethal temperature for the mycelium was 55 ℃ for 10 minutes. The optimal temperature for spore production was 28～40 ℃, with the most suitable temperature being 32 ℃. Light inhibited spore production, and the optimal pH for spore production was 6～8, with the most suitable pH being 8. The most suitable carbon and nitrogen sources for spore production were sucrose and gelatin, respectively. The lethal temperature for spores was 60 ℃ for 10 minutes. [Conclusion] *A. tubingensis* was causal agent of brown leaf spot on *D. cambodiana* in Mengzi, Yunnan and Urumqi, Xinjiang. Except for light and nitrogen sources, temperature, pH value, and carbon sources all had varying effects on mycelial growth and spore production. The pathogen grows and sporulates best between 22～34 ℃. It is recommended to strengthen control measures for brown leaf spot in the *D. cambodiana*.

Keywords: *Dracaena cambodiana*, Brown leaf spot, *Aspergillus tubingensis*, Phylogenetic analysis, Sporulation

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